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Review Article

Endothelium and Angiogenesis in Vasculature: Effect of Homocysteine and Folic acid

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Abstract

Endothelium is a highly efficient part of the vasculature that plays a very important role in fulfilling the metabolic requirements of the body. Endothelium dysfunction signified by the impaired activities of the endothelium leads to various acute diseases and symptomatic problems. One major role of the endothelial cells is angiogenesis, which either gets impaired or exaggerated in conditions like cardiovascular disorders and cancer, to name a few. Various genetic factors and signalling molecules also contribute to these impairments in the vasculature. Homocysteine is one such molecule which is the precursor of methionine generation pathway in the body. But it has been found that this molecule when tends to accumulate in the body due to certain inhibitions in its pathway, results into various problems associated with the endothelium and angiogenesis.

In this article the author has reviewed the functions of endothelium, the role of angiogenesis in vasculature, its role in the cardiovascular disorder especially atherosclerosis and the various consequences of hyper homocystenemia on the endothelium. The author also proposes the role of folic acid in mitigating the problems that arise due to homocysteine accumulation in the body, leading to various complications.

Keywords: Angiogenesis; Atherosclerosis; Endothelium; Folic acid; Homocysteine; Vasculature

Introduction

Angiogenesis and Vasculature

The vascular system in higher organisms evolved to transport oxygen, nutrients, growth factors, waste and immune cells across the organs in the body. Under metabolic stress conditions, the pre-existing cells of the vascular system signals the quiescent endothelial cells to initiate angiogenesis, a process that is defined as the growth of new blood vessels from existing one in the vasculature in order to support the tissue for healthy growth and metabolism [1,2].

Under normal, healthy conditions, endothelial cells remain quiescent under a balance of pro- and anti-angiogenic factors. When this equilibrium is disturbed and pro-angiogenic factors dominate, endothelial cells quickly take over the angiogenic phenotypes as either migratory tip cells or proliferating stalk cells [3,4]. Existing vasculature chooses the tip cells and initiates them to proceed towards the angiogenic signal [5]. Stalk cells proliferate behind the tip cell and extend the vascular lumen as sprouts elongate. Differentiation of the tip and stalk cells is tightly regulated by a feedback system that involves the Vascular Endothelial Growth Factor (VEGF), the Notch, and other genetic control signals. The mobilization of endothelial tip cells and expression of Delta-Like Ligand 4 (DLL4) is induced by VEGF. Activation of Notch signalling in neighbouring cells is mediated by DLL4 that suppresses their expression of VEGF receptor 2 (VEGFR2) and, promotes the stalk cell phenotype [6]. Endothelial cells keep switching phenotypes during angiogenesis, depending on their fitness as the tip cell [6]. By ensuring that the most suitable endothelial cells are in the tip and stalk positions, the DLL4–Notch feedback system promotes efficient sprouting and mediates vascular growth patterns [7]. However, metabolic requirements of endothelial cells during angiogenesis are under-investigated. The mechanisms of endothelial cells (such as Notch signalling) that differentially regulate their metabolic state remain unclear (Figure 1).

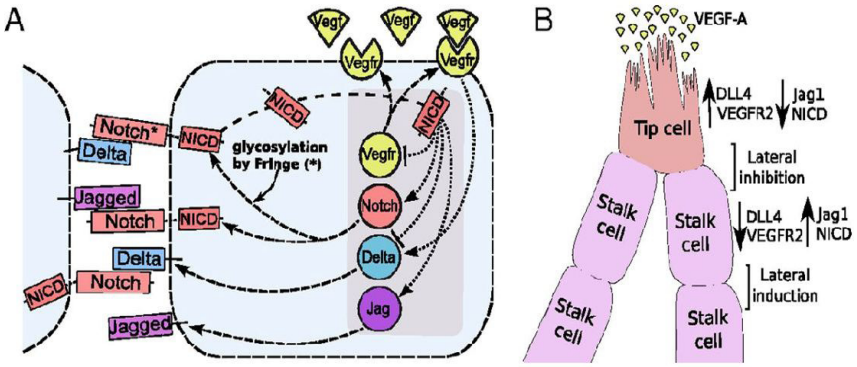

Figure 1: Overview of the intracellular and intercellular interplay between Notch and VEGF signalling pathways.

(A) Notch signalling is activated when the transmembrane receptor of one cell (Notch) binds to the transmembrane ligand (Delta or Jagged) of the neighbouring cell (trans-activation). This trans-activation cleaves Notch to produce Notch Intracellular Domain (NICD) that is released in the cytoplasm and then enters the nucleus to modulate the transcription of many target genes. NICD can activate Notch and Jagged and inhibit Delta and VEGF Receptor 2 (VEGFR2). Glycosylation of Notch by Fringe modifies Notch to have a higher affinity for binding to Delta and a lower affinity for binding to Jagged. Interaction between Notch receptor and ligands (Delta or Jagged) of the same cell (cis-inhibition) leads to the degradation of both the receptor and the ligand; thus, no NICD is generated. VEGF-A binds to VEGFR2, thus activating VEGF signalling in the cell that activates Delta (DLL4).

(B) Cells with high levels of Delta, VEGFR2, and active VEGF signalling develop filopodia and migrate toward the VEGF-A gradient, leading the formation of the new branch and are called tip cells. DLL4 from tip cells inhibits the neighbouring cells to also adopt a tip phenotype, thereby forcing them to adopt the stalk fate (low Dll4, high Jagged1, and NICD). Stalk cells, by virtue of the lateral induction characteristics of Notch-Jagged signalling, can induce neighbouring cells to adopt a stalk cell, therefore elongating the lumen [8].

The endothelium is a single layer of Endothelial Cells (ECs) that lines the blood vessel lumen and controls the various functions of the vasculature. It regulation of vascular tone and helps in leukocyte trafficking, blood coagulation, nutrient and electrolyte uptake, leads to the neovascularization of hypoxic tissue, and many more [4]. Disturbance in the physiological function of the endothelium (a condition termed EC dysfunction) contributes to cardiovascular disease and diabetes whereas diseases such as cancer and age-related macula degeneration are characterized by new blood vessel formation (a process termed angiogenesis) [10]. Targeting the factors leading to endothelium dysfunction or inhibiting the pathological angiogenesis is potentially beneficial for a wide variety of diseases, but current therapies focus primarily on growth factors, receptors, signalling molecules and thus have limited efficacy or specificity.

An emerging but understudied therapeutic target is EC metabolism and the factors which if present in concentrated leads to endothelial dysfunction through various metabolic pathways. It has been long known that risk factors for cardiovascular disease (hypercholesterolemia, hypertension, dyslipidemia, diabetes, obesity and aging) cause EC-specific metabolic perturbations leading to EC dysfunction. Similarly, the links between EC metabolism and angiogenesis are apparent as angiogenic ECs migrate and proliferate in metabolically challenging environments such as hypoxic and nutrient-deprived tissue [11].

Endothelium in Normal Vascular Homeostasis

The healthy endothelium responds well to physical and chemical signals by production of a wide range of factors that regulate vascular tone, cellular adhesion, thrombo-resistance, smooth muscle cell proliferation, and vessel wall inflammation. The endothelium plays a vital role in maintenance of vascular tone by production and release of several vasoactive molecules that relax or constrict the vessel, and also by responding to or by modifying the circulating vasoactive mediators such as bradykinin and thrombin. This vasomotion plays a direct role in the balance of tissue oxygen supply and metabolic demand and is also involved in the re-modelling of vascular structure and long-term organ perfusion [12].

Furchgott and Zawadzki, (1980) [13] were the first to demonstrate the existence of an endothelium-derived relaxing factor that was subsequently shown to be nitric oxide (NO). NO is generated from L-arginine by the action of endothelial NO Synthase (eNOS) in the presence of cofactors such as tetra hydro biopterin [14]. NO diffuses to the vascular smooth muscle cells and activates guanylate cyclase, which leads to cGMP-mediated vasodilatation. Shear stress is a key activator of eNOS in normal physiology, and this adapts organ perfusion to changes in cardiac output [15]. eNOS may also be activated by signalling molecules such as bradykinin, adenosine, vascular endothelial growth factor (in response to hypoxia), and serotonin (released during platelet aggregation) [16]. The endothelium also mediates hyperpolarization of vascular smooth muscle cells via an NO-independent pathway,

which increases potassium conductance and subsequent propagation of depolarization in vascular smooth muscle cells, to maintain vasodilator tone [17]. The endothelium-derived hyperpolarizing factors involved in this process are only partially understood (such as the cytochrome-derived factors and possibly C-type natriuretic peptide), and may differ between vascular beds. However, it is well recognized that Endothelium-Derived Hyperpolarizing Factor can compensate for loss of NO-mediated vasodilator tone, particularly in the microcirculation, and this appears important when NO bioavailability is reduced [18].

The endothelium modulates vasomotion, not only by release of vasodilator substances, but also by an increase in constrictor tone via generation of endothelin and vasoconstrictor prostanoids, as well as via conversion of angiotensin I to angiotensin II at the endothelial surface [19,20] . These vasoconstrictor agents predominantly act locally, but may also exert some systemic effects and have a role in the regulation of arterial structure and remodelling.

Under healthy vascular physiology, NO plays a key role to maintain the vascular wall in an original state by inhibition of inflammation, cellular proliferation, and thrombosis. This is in part achieved by s-nitrosylation of cysteine residues in a wide range of proteins, which reduces their biological activity [21]. The target proteins include the transcription factor NFκB, cell cycle–controlling proteins, and proteins involved in generation of tissue factor [22]. Furthermore, No limits oxidative phosphorylation in mitochondria [23]. Laminar shear stress is probably the major factor that maintains this quiescent, NO-dominated, endothelial phenotype [24].

Endothelium and Angiogenesis in Atherosclerosis

Large human arteries contain an adventitious layer called vasavasorum in their microvasculature. Normal vasavasorum originate from coronary artery branch points at regular intervals and run longitudinally along the vessel wall (first order vasavasorum). Some layers of these vasavasorum separate to form circumferential arches around the main coronary lumen (second order vasavasorum). Because diffusion of blood nutrients from the lumen is limited to a distance of ≈100 μm, a primary function of these vessels is thought to be the transport of nutrients to the vessel wall, although other roles are not precluded [25]. Association between intimal neovascularization and atherosclerosis was first noted by Koester [26] in 1876; followed by similar observations of Winternitz and co-workers [27] in 1938. Rupture of plaque capillaries could trigger intraplaque hemorrhage, leading to coronary thrombosis was first proposed by Patterson [28] in 1938. Later it was found that the intimas of adult human arteries remain a vascular in their role until they reach a certain thickness. Proliferation of the adventitial vasculature of coronary arteries allows atherosclerotic plaques to develop beyond a critical thickness by supplying oxygen and nutrients to the core of the lesions. Barger and Beeuwkes [29] subsequently proposed that the neovascular network in coronary atherosclerotic plaques may be more fragile and prone to rupture and therefore may lead to plaque destabilization and vascular spasm, resulting into acute coronary syndromes. Subsequent works established an association between neovascularization and atherosclerosis showing a correlation between the extent of atherosclerosis and plaque neovascularization in human pathological samples, and in the coronary arteries of hypercholesterolemic primates. Williams et al., [30] found that atherosclerosis in hypercholesterolemic monkeys increases blood flow through the vasavasorum, whereas plaque regression induced by withdrawal of a high cholesterol diet was associated with loss of vasavasorum and a marked decrease in blood flow through the vasavasorum to the coronary intima and media. Later a more complex picture of the relationship between plaque neovascularization and atherosclerotic pathology was developed and it was found that neovascularization is most common at sites of infiltration by chronic inflammatory cells such as macrophages and lymphocytes but less common in highly calcified or hyalinised plaques.

The relationship between neovascularization and the severity of disease is less certain, but one study [31] that used micro CT found a highly significant correlation (r=0.71) between the number of vasavasorum and wall area in hyper cholesterolemic porcine coronary arteries. A role for neo-vascularization in plaque instability has been widely hypothesized, but direct evidence for it is lacking, partly because the critical factors that precipitate plaque rupture remain largely unknown and partly because reliable animal models of plaque rupture analogous to the human situation have not yet been developed.

Nevertheless, studies in human lesions suggest that a spatiotemporal relationship exists between micro vessels and the regions of plaques that are highly vulnerable to rupture. Studies [32-34] on neovascularization in advanced human atherosclerotic plaques concluded that micro vessel formation is strongly correlated with both plaque rupture and the signature features of vulnerable plaques. Thus, an increase in micro vessel density occurred in ruptured plaques as compared to the non-ruptured ones and this increase in vessel density was also found to be strongly associated with a high degree of macrophage infiltration, intra plaque hemorrhage, and thin cap lesions.

Role of Endothelial Progenitor Cells

Endothelial Progenitor Cells (EPCs) have been widely assumed to hold immense promise for the treatment of coronary artery disease, largely because of their ability to regenerate endothelial cells after angioplasty and their potential for the revascularization of ischemic tissue. However, it was seen that both circulating and bone marrow-derived EPCs and stem cells are a

major source of lesion associated VSMCs, endothelial cells, and plaque micro vessels in mouse models of transplant atherosclerosis [35-37]. Experimental evidences in favor of a role of both angiogenesis and EPC in atherosclerosis has come from mouse models-in the case of EPCs, largely models of allograft atherosclerosis, whereas several studies support the use of both VEGF and EPCs for re-endothelialization of balloon injured arteries or for the seeding of prosthetic grafts and stents [38-40]. The findings that EPCs may contribute to atherosclerotic plaque formation, seems likely that the momentum currently building behind the medical use of EPC and stem cells in general will lead to clinical trials for cardiovascular disease in the foreseeable future.

Homocysteine Biosynthesis and Biochemical Roles

Homocysteine is not obtained from the diet. Instead, it is biosynthesized from methionine via a multi-step process. First, methionine receives an adenosine group from ATP, a reaction catalyzed by S-Adenosyl-Methionine Synthetase, to give S-Adenosyl Methionine (SAM) [41,42]. The adenosine is then hydrolyzed to yield L-homocysteine, which has two primary fates: conversion back into L-methionine (remethylation pathway) using Tetra Hydro Folate (THF, asmethyl donor) and cobalamin-related enzymes or to L-cysteine (trans-sulfuration pathway, which is quite common in mammals). Cystathionine β-synthase catalyzes the condensation of homocysteine and serine to give cystathionine. This reaction uses pyridoxine (vitamin B6) as a cofactor [43]. Cystathionine γ-lyase then converts cystathionine to cysteine, ammonia, and α-ketobutyrate. Bacteria and plants follow different pathway to produce cysteine, relying on O-acetylserine. Homocysteine can also be recycled to give homocysteine thiolacetone, a five-membered heterocycle. Because of this "self-looping" reaction, homocysteine-containing peptides tend to cleave themselves thereby generating oxidative stress. Since homocysteine is the intermediate in most of the metabolic pathways, it directly or indirectly impacts all methyl and sulfur group metabolism occurring in the body. Experiments show that if a high level of homocysteine and adenosine accumulates in the cell, all methylation reactions are inhibited [44]. The re-methylation pathway results in the transfer of methyl group (CH3) to homocysteine by either methycobalamin or betaine (trimethyglycine). Methylcobalamin originally receives its methyl group from S-Adenosyl Methionine (SAM) or S-methyl Tetra Hydro Folate (S-methyl THF), an active form of folic acid. After re-methylation, methionine can be reutilized to produce SAM, the body's universal methyl donor, which participates in key metabolic pathways, including methylation of DNA and myelin, synthesis of carnitine, coenzyme Q10, creatine, epinephrine, melatonin, methylcobalamin and phosphatidylcholine as well as methyl detoxification reactions [45].

The trans-sulfuration pathway of methionine/homocysteine degradation produces the amino acids cysteine and taurine which are important nutrients for cardiac health, hepatic detoxification, cholesterol excretion, bile salt formation and glutathione production. This pathway is dependent on adequate dietary intake and hepatic conversion of Vit B6 into its active form, P5P (Pyridoxal 5'- phosphate, Vit B6). Amino acid serine is generated from betaine via the homocysteine-re-methylation pathway [41].

Hcy is methylated to form the essential amino acid methionine in two pathways. The reaction of Hcy methylation catalyzed by Vitamin B12- dependent methionine synthase captures a methyl group from the folate-dependent one carbon pool (5-methyl tetrahydrofolate) [5]. A second pathway requires betaine (N,N,N-tetramethylglycine) as a methyl donor for the methylation of homocysteine catalyzed by betaine homocysteine methyltransferase. The catabolic pathway of homocysteine, known as the transsulfuration pathway converts homocysteine to the amino acid cysteine via two Vitamin B6-dependent enzymes; Cystathionine β-synthase (CβS) and Cystathionase. CBS catalyzes the condensation of homocysteine with serine to form cystathionine which is further converted to cysteine, α-ketobutyrate and ammonia by Cyatathionase [43,44] (Figure 3).

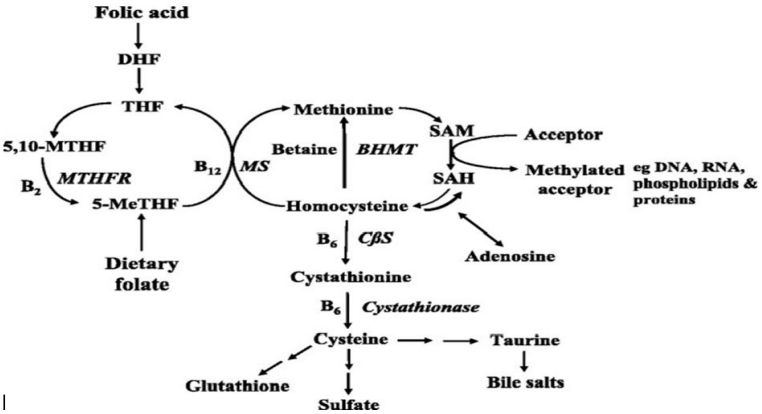

Figure 3: Metabolism of folate and homocysteine. AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; B6, vitamin B6 (pyridoxal phosphate); B12, vitamin B12 (methylcobalamin); CBS, cystathionine β-synthase; MS, methionine synthase; MTHFR, methylenetetrahydrofolate

reductase; THF, tetrahydrofolate (Source: American Journal of Physiology- Heart and Circulatory Physiology Published 1 July 2004 Vol. 287 no. 1: H91-H99) [46].

Aetiology of Hyper Homo Cysteinemia (HHC)

The normal range of HCY levels is between 5 and 15 µmol/L. Fasting values for moderate HHC are usually defined as 16 to 100 µmol/L and severe HHC is defined as greater than 100 µmol/L. Variety of factors influence the plasma level of HCY in humans including nutritional deficiencies in vitamin cofactors, certain genetic polymorphisms and some medications. Deficiencies in enzyme co-factors (folate, vitamin B12, vitamin B6) are associated with many cases of elevated HCY. Thus, it has been postulated that elevated HCY may be a marker for vitamin deficiency [44,45]. Homocysteine levels in the plasma are controlled by its conversion to other metabolites via one of two pathways: the transsulphation pathway or remethylation pathway. Transsulfuration is mediated by the Vitamin B6 dependent enzyme Cystathione-B-Sythase (CBS). Remethylation is catalyzed by methionine synthase and depends on vitamin B12. The latter reaction also depends on the donation of a methyl group created in the interaction between Methylene Tetra Hydro Folate Reductase (MTHFR) and Dietary Folate (figure 2) [41]. The enzyme MTHFR is coded by the gene with the symbol MTHFR on chromosome 1 location p36.3 in humans. There are DNA sequence variants (genetic polymorphisms) associated with this gene. Two of the most investigated are C677T (rs1801133) and A1298C (rs1801131) Single Nucleotide Polymorphisms (SNP) [47].

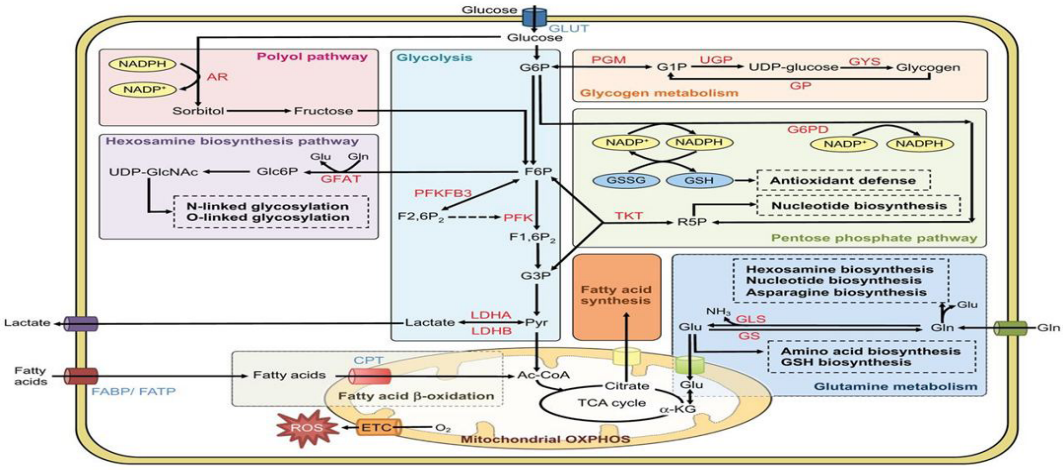

Figure 2: Endothelial cell metabolism. A simplified map of the known metabolic pathways in endothelial cells and the enzymes involved. Dashed arrow indicates allosteric activation. α-KG, α-ketoglutarate; Ac-CoA, acetyl coenzyme A; AR, aldolase reductase; CPT, carnitine palmitoyltransferase; ETC, electron transport chain; F1,6P2, fructose-1,6-bisphosphate; F2,6P2, fructose-2,6-bisphosphate; F6P, fructose-6-phosphate; FABP, fatty acid binding protein; FATP, fatty acid transport protein; G1P, glucose-1-phosphate; G3P, glyceraldehyde-3-phosphate; G6P, glucose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; GFAT, glutamine fructose-6-phosphate amino-transaminase; Glc6P, glucosamine-6-phosphate; Gln, glutamine; GLS, glutaminase; Glu, glutamate; GP, glycogen phosphorylase; GLUT, glucose transporter; GS, glutamine synthetase; GSH, glutathione; GSSG, glutathione disulfite; GYS, glycogen synthase; LDH, lactate dehydrogenase; OXPHOS, oxidative phosphorylation; PFK, phosphor fructokinase; PFKFB3, phosphofructokinase-2/fructose-2,6-bisphosphatase isoform 3; PGM, phosphor glucomutase; Pyr, pyruvate; R5P, ribose-5-phosphate; ROS, reactive oxygen species; TKT, transketolase; TCA, tricarboxylic acid; UDP-GlcNAc, uridine diphosphate-N-acetylglucosamine; UDP-glucose, uridine diphosphate glucose; UGP, UDP-glucose phosphorylase [9].

The C677T polymorphism in the MTHFR enzyme has been implicated in cases of moderately elevated HCY. The defective genetic code produces a thermolabile form of the MTHFR enzyme and has a population frequency of around 10 percent [48]. This polymorphism is a C-T transition at nucleotide 677, causing an alanine to valine substitution [12]. Individuals with the MTHFR 677TT genotype are at a higher risk of Coronary Heart Disease (CHD), particularly in the presence of low folate levels [49].

However, studies have shown that high plasma HCY levels in patients with the thermolabile variant only occur in those with low serum folate levels [50,51] Not surprisingly, some drugs with anti-folate properties, such as methotrexate and trimethoprim, have been associated with HHC [52,53]. Many mechanisms have been postulated by which HCY may cause vascular injury. HCY-induced atherosclerosis is the result of endothelial dysfunction and injury followed by platelet activation and thrombus formation [54]. The proposed mechanisms by which HCY causes this vascular injury and subsequent atheroma/thrombus formation include the promotion of oxidative stress, leukocyte recruitment, foam cell production, smooth muscle and collagen proliferation, marked platelet

accumulation and impaired nitric oxide production. HCY is auto-oxidized when added to plasma and produces reactive oxygen species (free radicals) such as superoxide and hydrogen peroxide, which have been implicated in the direct injury of endothelial cells [55-58]. This free radical-induced injury may expose underlying collagen and smooth muscle cells, which proliferate and promote the activation of platelets and leukocyte recruitment [59,60]. A by-product of HCY's auto-oxidation is an HCY thiolactone complex, which can combine with LDL-cholesterol, forming an aggregate that is engulfed by vascular macrophages [61]. This new lipid-laden macrophage is called a foam cell, which then releases its lipid into the atherosclerotic plaque [62]. This increases the size and instability of the plaque. Several studies have shown that HCY impairs the production of nitric oxide, an endogenous vasodilator [55,63]. This may contribute to impaired endothelium-dependent vasodilation, which would further lead the development of vascular injury and atherosclerosis.

Hcy Increases Intracellular ROS Levels and Induces Apoptosis in Endothelial Cells

An elevated level of Homocysteine (Hcy) limits the growth and induces apoptosis. However, the mechanism of Hcy-induced programmed cell death in endothelial cells is largely unknown. Studies [64] using rat heart Micro Vascular Endothelial Cells (MVEC) treated with different doses of Hcy at different time intervals, followed by measurement of apoptosis and ROS generation have proved that Hcy induces intracellular Reactive Oxygen Species (ROS) production that leads to the loss of transmembrane mitochondrial potential ($\Delta\psi m$) accompanied by the release of cytochrome-c from mitochondria. Cytochrome-c release contributes to caspase activation, such as caspase-9, caspase-6, and caspase-3, which results in the degradation of numerous nuclear proteins including poly (ADP-ribose) polymerase (PARP), which subsequently leads to the inter nucleosomal cleavage of DNA, resulting cell death. Another study [65] evaluated whether reactive oxygen species (ROS)-producing signalling pathways contribute to Hcy-induced apoptosis induction, with specific emphasis on NADPH oxidases. They used Human umbilical vein endothelial cells and determined the effect of Hcy on caspase-3 activity, annexin V positivity, intracellular NOX1, NOX2, NOX4, and p47 (phox) expression and localization, nuclear nitrotyrosine accumulation, and mitochondrial membrane potential ($\Delta\Psi m$). They found that Hcy induces caspase-3 activity and apoptosis in a concentration dependent manner. These researchers have also shown that Hcy causes endothelial cell apoptosis in part by generating ROS and decreasing membrane potential. This in turn released cytochrome-c and activated the intracellular caspase-proteolytic cascades especially the activation of caspase 9 and caspase 3, degradation of numerous nuclear proteins including poly (ADP-ribose) Polymerase (PARP) resulting in nuclear fragmentation and cell death.

Hcy Impairs Angiogenesis

Homocysteine is a risk factor for the development of coronary artery disease [66,67]. Laboratory studies suggest that an elevated homocysteine concentration is both atherogenic and thrombogenic [42]. There may be several possible mechanisms by which hyperhomocysteinemia impair angiogenesis. First, hyperhomocysteinemia-induced endothelial dysfunction may account for the impaired angiogenesis. Homocysteine reduces endothelium-dependent vasodilatation by elevating plasma levels of asymmetric dimethylarginine, a potent inhibitor of Nitric Oxide (NO) synthase [68]. Homocysteine impairs endothelium derived NO formation, not only in large conduit arteries, but also in microvessels in vivo. Endothelium-derived NO is an important regulator of angiogenesis. For example, endothelium-derived NO maintains endothelial cell integrity and the expression of integrin v-3, thus promoting endothelial podokinesis and migration [69,70]. Angiogenesis induced by vascular endothelial growth factor was attenuated by inhibitors of NO synthase [71]. Second, hyperhomocysteinemia-induced production of reactive oxygen radicals may contribute to further impairment of angiogenesis (Loscalzo, 1996)72. Enhanced generation of oxygen radicals in the hyper homo cysteinemia state might further degrade NO. Third, homocysteine itself might directly inhibit endothelial cell proliferation and/or migration [73]. Outinen et al. demonstrated that homocysteine induced arrested growth in human endothelial cells in vitro [74]. Taken together, endothelial dysfunction, decreased NO bioactivity, and increased oxidative stress seem to account for impaired angiogenesis in the hyper homo cysteinemia state in vivo.

Folic Acid Alleviates the Physiological Changes Induced by Hcy

As shown in the folate and homocysteine pathway (figure 3), folic acid plays a pivotal role in regulating the concentration of homocysteine, which is further degraded to various other intermediates through different pathways. Circulating homocysteine can be increased by genetic deficiency of enzymatic pathways involved in its catabolism as well as by environmental factors including nutritional deficiencies, lifestyle, physiological conditions, drugs and some diseases, which mainly induce deficiency of folic acid, vitamin B12 and B6. Therefore, plasma homocysteine can be reduced by interventional therapy with folic acid and vitamin B12, 13. Hyper homo cysteinaemia is an independent risk factor of CAD [75]. Hyperhomocysteinaemia may cause injury to the endodermis of the vessels, activate the platelets, improve the congregation of the platelets, enhance the production of fibrinogen, and promote migration and proliferation of smooth muscle cells. Hcy can also activate protein kinase C, promote the expression of c-fos and c-myb genes in vascular smooth muscle cells 61,76. Long term folic acid treatment improves arterial endothelial function and has potential

implications for the prevention of atherosclerosis in adults with hyper homo cysteinaemia [77-79]. The first prospective randomized placebo-controlled intervention study suggested that coronary endothelial function improves after treatment with folic acid and cobalamin [80]. Folic acid significantly improves endothelial function in otherwise healthy cigarette smokers and during pregnancy [81]. Hcy exerts atherogenic effects by enhancing chemokine responses in cells involved in atherogenesis and folic acid supplementation may down regulate these inflammatory responses [82]. Folic acid supplementation to hyperhomocysteinaemic subjects resulted in a decrease in total blood homocysteine concentrations; moreover, there was a tendency to reverse the coagulation status and oxidative stress [83].

Conclusion

Endothelial dysfunction is a major contributor to atherosclerosis and a variety of other cardiovascular diseases. It has been shown that homocysteine, a sulfur-containing amino acid, is a metabolite of the essential amino acid methionine, and exists at a critical biochemical intersection in the methionine cycle-between S-adenosylmethionine, the indispensable ubiquitous methyl donor, and vitamins B12 and folic acid. High blood levels of homocysteine signal a breakdown in this vital process, resulting in far-reaching biochemical and life consequences. Homocysteine has been found to impair angiogenesis which downstream leads to necrosis and death of the vital regions of the myocardium. Hcy also infduces apoptosis of endothelial cells thereby exaggerating the consequences related to endothelial dysfunction and major part in this pathway is played by increase in ROS generation. Decreasing plasma total homocysteine by providing nutritional cofactors especially folic acid, for its metabolism has been shown to reduce the risk of cardiovascular events.

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